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USABILITY OF THE RESOURCES EXPERT WORKING GROUP FINAL REPORT

RECOMMENDATIONS FROM CYCLE 1 REVIEW

**PREPARED FOR THE
EEM TECHNICAL MANAGEMENT COMMITTEE**

EEM/1997/4

Canada

Publications relating to Environmental Effects Monitoring (EEM):

Aquatic Environmental Effects Monitoring Requirements (revised EPS 1/RM/18). Environment Canada. 1997. EEM/1997/1

Pulp and Paper Aquatic Environmental Effects Monitoring Requirements.
Environment Canada. 1997. Annex 1 to EEM/1997/1

Tracer Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/2 is also published as: Ali, N et al, 1997. Exposed or not exposed: the effectiveness of using tracers to verify exposure of fish to pulp and paper mill effluent. Canadian Technical Report of Fisheries and Aquatic Sciences 2162.

Dioxin Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/3

Usability Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/4

Toxicology Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/5

Fish Survey Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/6

Benthic Community Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/7

Lowell, R.B. Discussion paper on critical effect size guidelines for EEM using benthic invertebrate communities. Report to the Environmental Effects Monitoring Program. EEM/1997/8

Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring. 1998. EEM/1998/1

Workshop on Integrated Approaches for Interpreting Environmental Effects Monitoring Data, Proceedings. EEM/1999/2

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Note

The contents of this report are the views of the members of the Environmental Effects Monitoring (EEM) Expert Working Group. As well, the recommendations in this report are the result of the Expert Working Group process and represent the conclusions of the Expert Working Group.

The recommendations and decision trees put forward in this report will be submitted to the EEM Technical Management Committee (TMC). This information will be used by the TMC to produce the TMC recommendations for updates to the regulatory requirements of the EEM program.

The documents which will contain the revised regulatory requirements will be called:

- Aquatic Environmental Effects Monitoring Requirements, EEM/1997/1
- Pulp and Paper Aquatic Environmental Effects Monitoring Requirements, ANNEX 1 to EEM/1997/1

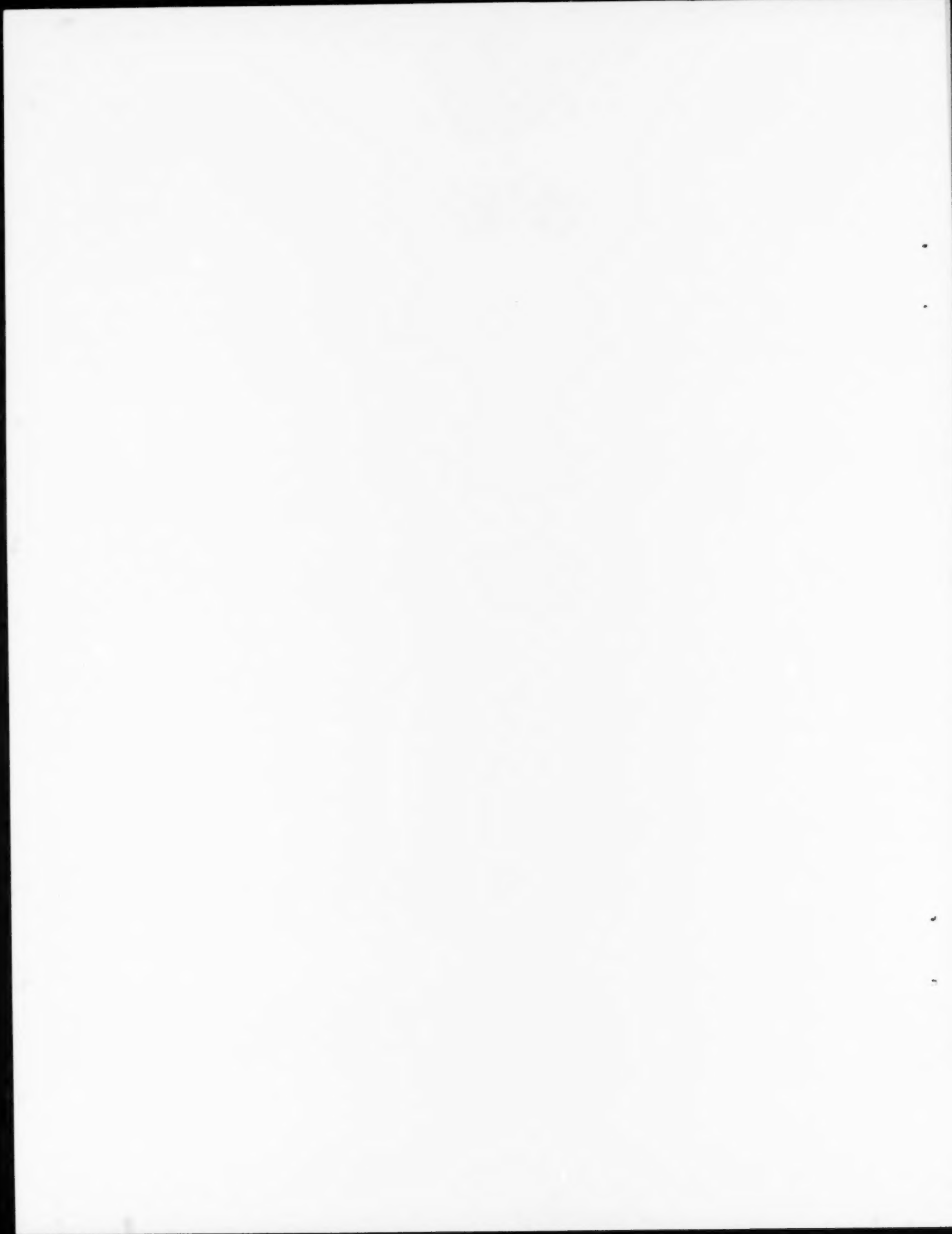
Acknowledgments

The EEM Technical Management Committee (TMC) extends its thanks to the members of the Expert Working Groups for their contribution to the EEM program. The members of the group have produced their reports in a very tight time frame and they have displayed a willingness to work together to meet common objectives.

The review performed by the Expert Working Groups and their recommendations for enhancements to the program are essential ensuring that the EEM program remains based on sound science.

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1. Introduction

In May 1992, amendments to the federal Pulp and Paper Effluents Regulations under the Fisheries Act required regulated pulp and paper mills to conduct environmental effects monitoring (EEM) studies. The objective of the EEM program is to determine whether indigenous fish, fish habitat and the utilization of the fisheries resources are adequately protected by the PPER effluent limits. Results for the first "baseline" monitoring Cycle have been reviewed to help determine the utility of the field and lab methodologies used and also to help gain an understanding of the natural variability of the local ecosystems.

Prior to the review of Cycle 1 data, there were also indications of some technical problems with certain methodologies. To help resolve some of these problems, government and industry representatives agreed to the need of forming expert working groups (EWG) to address the specific areas of difficulty. Consequently, seven EWG were formed over the past year to address technical issues in the following areas:

- tracers
- fish population assessment
- benthic invertebrate community assessment
- toxicology
- dioxins and furans
- fish tainting (usability of the resources)
- information management

The purpose of this report is to discuss the findings of Cycle 1 pertaining to the usability of the resource ("tainting evaluation") component of EEM and to recommend improvements for future monitoring Cycles.

1.1 The Usability of the Resources EWG: Objectives and Process

The Usability of the Resources EWG was formed in October, 1996. The group was composed of science advisors and scientists from Environment Canada, Fisheries and Oceans, the Pulp and Paper Industry and the consulting community

The group members include:

Valerie Naish (Co-Chair) Centre Innovation Domtar, Ste. Anne de Bellevue
Ed Porter (Co-Chair) Environment Canada, Hull
Alan Redenbach, Environment Canada, Vancouver
Hélène Dupuis, Fisheries and Oceans, Moncton
Roberta York, Fisheries and Oceans, Winnipeg
David Graham, Weyerhaeuser Canada Ltd., Grande Prairie

Jelena Grabovac, Eurocan Pulp and Paper, Kitimat
Sharon Gibbons, Paprican, Pointe Claire
Phil Whitney, Weldwood of Canada Ltd., Hinton
Dennis Farara, Beak International Inc., Brampton
Michael Gordon, M.R. Gordon and Associates Ltd., Halfmoon Bay
Glenn Atkinson, SCS Consultants, Ottawa

The general objectives of the group were (refer to Appendix A for the Terms of Reference):

- to review the concept and purpose of the tainting evaluation requirement
- to identify the difficulties encountered in meeting this program requirement
- to indicate situations where the requirement has been successfully met
- to take into account situations where the national monitoring approach for Cycle 1 was unsuccessful and to provide alternative approaches that will address these site-specific difficulties
- to provide a report identifying potential alternative approaches and areas requiring further research.

Specific issues to address were summarized under five general areas:

- Triggers to implement Taint Testing
- Fish Exposure Conditions and Methods
- Fish Capture, Preparation and Preservation in the Field
- Fish Preparation in the Laboratory
- Sensory Evaluation Panels and Methods

The group activities included meetings and teleconferences as well as the review and preparation of technical documentation in the various areas listed above. The duration of the group activities was from October 1996 to April 1997.

2. Review of Cycle 1

Six mills conducted tainting evaluations in Cycle 1 of the EEM program - two from Alberta, three from British Columbia and one from Nova Scotia.

Key elements of the Cycle 1 tainting studies i.e. the trigger for the study (complaints), fish species used, exposure conditions, and sensory test information are summarized in Table 1 and discussed briefly below.

The need to conduct a tainting evaluation was judged on a site-specific basis, triggered by a well-documented record of complaint or creel records indicating that an area was no longer fished due to anecdotal fish tainting information. In Cycle 1, these trigger criteria were applied at mills with well established secondary treatment systems i.e. mainly the mills in western Canada. The majority of mills in Eastern Canada (particularly Ontario and Quebec regions) were in the construction phase of secondary treatment installation during Cycle 1.

There may have been inconsistencies when deciding to initiate tainting evaluations. There may also have been different interpretations of the meaning of "well-documented record of complaint" between sites, particularly with respect to historical versus recent complaints and the intent of the wording "well-documented record".

The mills conducting the Cycle 1 studies followed the guidance provided in the Annex 1 and the Technical Guidance Document. For example, the majority of mills used indigenous species from the reference and exposure sites for tainting evaluations. Also, some form of triangle test was used by all mills for the sensory evaluation; the preference, likeability and difference-from-control tests were used in some studies.

In general, the results from Cycle 1 were inconclusive in that there were confounding factors which precluded the definite determination of whether or not the mill was responsible for the flavour difference detected.

The most common confounding factors identified in the review of study designs were as follows:

- fish populations from reference and exposure sites were not entirely isolated from each other e.g. in the Crestbrook study.
- reference and exposure fish were from different habitats and captured by different techniques e.g. Weyerhaeuser, Alberta study where reference fish were taken from a lake by commercial fishermen and the exposure area fish were collected in the river downstream of the mill with electrofishing gear.
- the influence of other point and non-point sources was not or could not be determined.

Table 1: SUMMARY OF CYCLE 1 STUDIES

Mill	Complaints		Species	Exposure Conditions	Source of Fish	Sensory Test**
	Historic	Recent*				
Weldwood, Hinton, AB	yes	no	Mountain White Fish	Indigenous	Exp-Athabasca R Ref-North Saskatchewan R	Triangle Preference Difference-from- Control
Weldwood, Hinton, AB			Mountain White Fish	Laboratory	Red Deer R	Triangle Preference Difference-from- Control
Weyerhaeuser, Grande Prairie, AB	yes	no	Walleye	Indigenous	Exp - Wapiti River Ref - South Buck & Losegun L.	Triangle Preference
Crestbrook, Skookumchuck BC	yes	no	Mountain White Fish	Indigenous	Kootenay R	Triangle Preference Likeability
Weyerhaeuser, Kamloops, BC	yes	-	Mountain White Fish	Indigenous	Exp - Thompson R. & Kamloops L. Ref - South Thompson R	Triangle
Weyerhaeuser, Kamloops, BC			Rainbow Trout	Indigenous	Exp - Thompson R. & Kamloops L. Ref - Little R. & Little Shuswap L.	Triangle Preference
Eurocan***, Kitimat, BC	yes	yes	Eulachon	Laboratory	Kemano R.	Triangle Acceptance
				Indigenous	Exp - Kitimat R. Ref - Kemano R.	
				In-situ cages	Kemano R.	
Eurocan***, Kitimat, BC	not applicable		Rainbow Trout	Laboratory In-situ cages	Hatchery	Triangle
Eurocan***, Kitimat, BC	no	no	Dolly Varden	Indigenous	Kitimat R.	Triangle
Scott Paper, Boat Harbour, NS	yes	yes	Lobster	Laboratory	Unknown (retail)	Triangle

*Comments solicited by consultants from local indigenous/fishermen during the Cycle 1 EEM study.

** Triangle tests included one or more of the following variations: balanced triangle, 3-alternative forced choice and a "double triangle".

***The Eurocan studies were done annually over a period of six years. Various methods were used to address a wide variety of objectives.

Methodological problems in the sensory tests used included:

- the panelists were not specifically trained to identify tainting caused by pulp and paper mill effluent.
- the triangle test is a very sensitive sensory test that can detect small differences which may or may not be due to effluent exposure.
- the triangle test is used to detect differences in taste but does not give an indication of the degree of difference.
- preference/likeability methods were used inappropriately as a measure of the presence and intensity of taint.

2.1 Discussion of Cycle 1 Issues

The EWG was able to come to consensus on recommendations for further guidance on the technical issues related to the tainting methodology. It is the recommendation of the group that Annex B, C and D of this report should replace the existing guidance in the Technical Guidance Document (TGD).

The issues related to the tainting evaluation component of the EEM program were not all technical in nature. Considerable discussion by the EWG focused on the following non-technical tainting evaluation issues:

Objective of the Tainting Evaluation in the EEM Program

The EWG consulted the TMC to obtain clarification of the original intent of tainting evaluations within Cycle 1. The TMC response indicated that tainting evaluation was included because it deals directly with the "use of the fish resource" however it was recognized as a site-specific component of the EEM program unlike the other core elements i.e. the adult fish survey, the benthic community study etc. Once the site-specific tainting issue is resolved by following tests procedures recommended in the Technical Guidance Document, then further tainting evaluation is not required.

Tainting Trigger

There were two views within the EWG concerning the consistency of the tainting trigger application: some group members believed that there was inconsistent application of the trigger evidenced by the fact that only six mills in Canada conducted tainting evaluations as part of Cycle 1. Other group members believed that the trigger was applied consistently but that there were different interpretations of the trigger wording among the various regions. The EWG agreed that the trigger should be re-worded so that the intent of the tainting trigger would be more clear. Recommended wording changes to Annex 1 are provided in the recommendation section of this report. Further guidance for trigger application is recommended for inclusion in the Technical Guidance Document. The suggested wording is provided in Appendix B of this report.

Interpretation of Tainting Results

The general conclusion of the Cycle 1 studies review was that the results were inconclusive. This was due to many confounding factors some of which were reference and exposure site selection, fish species selection, exposure regime and non-standardized sensory methods used. The EWG has recommended standardization of the tainting methodology from fish exposure to laboratory preparation techniques for sensory evaluation as described above. However it is the view of the group members that there will be instances where the results of future tainting evaluations may continue to be inconclusive. This is due to the restrictive exposure and methodological criteria that must be met in order to provide statistically meaningful data and to the fact that some mills will not be able to meet these criteria. In many cases, conducting a tainting evaluation will only provide results that will be added to the existing information and local knowledge i.e. as additional weight of evidence, to help in assessing a site-specific situation.

2.2 Conclusions

Further guidance is needed with respect to the criteria to trigger a site-specific tainting study.

Further guidance is needed with respect to the tainting methodology in order to eliminate the potential for confounding factors and inconclusive results. The specific issues requiring guidance are:

- Fish Exposure Conditions and Methods
- Fish Capture, Preparation and Preservation in the Field
- Fish Preparation in the Laboratory
- Sensory Evaluation Panels and Methods

3. Recommendations

The assessment of effects on use of fisheries resources is composed of two requirements: fish tissue analyses for dioxin and furan congeners and tainting evaluation. This section briefly summarizes the main recommendations of the EWG dealing with tainting evaluation (the dioxin EWG was formed to specifically address the dioxin monitoring requirement). The recommendations fall into two general categories: recommendations relating to triggers to commence tainting evaluation and recommendations relating to sensory evaluation approaches and methodologies.

3.1 Recommendations for triggers for sensory evaluation

One of the main issues raised during the EWG preliminary discussions was the need to address the original trigger requiring tainting evaluations. Unlike the other "core" monitoring requirements, the need to conduct tainting evaluation was determined on a site-specific basis. Consequently, "triggers" to commence tainting evaluation were prescribed in Annex 1 as follows:

"Where the results of fish contaminant analyses indicate there is no potential health hazard, and where there is a well-documented record of complaint in provincial or federal fisheries offices, or if creel records indicate areas of the receiving environment are not fished due to anecdotal information of fish tainting, a fish tainting evaluation must be conducted."

Many EWG members felt this wording was too ambiguous to be useful as a trigger to implement tainting evaluation where required, especially in terms of what is meant by "well-documented", the reliability of creel records and the time-frame from which complaints were considered to be relevant.

Recommendation 1: Section 9.2 of Annex 1 be revised to provide clearer "triggers" for the need to conduct tainting evaluation. The suggested wording is as follows:

"A mill need not conduct a fish tainting evaluation if at least one of the following conditions is met:

- fish contaminant analysis demonstrates that there is a potential health hazard, or
- there is no well documented record of complaint ¹since 1992 or,
- a sensory evaluation conducted in the previous EEM Cycle has demonstrated that tainting is no longer an issue.

¹ A well documented complaint can include (but need not be limited to) written complaints, fax or e-mail, documented telephone or verbal complaints to the mill or to government agencies.

Notwithstanding the above, each mill must conduct a fish tainting evaluation if fish contaminant analysis demonstrates that there is no potential health hazard and at least one of the following conditions is met:

- a sensory evaluation conducted in the last EEM Cycle has demonstrated that tainting is an issue, or
- there is a new record of complaint since the last EEM sensory evaluation.

The EEM study design for each mill should include a review of all pertinent information relative to tainting and an assessment of whether a tainting study is required in the current EEM Cycle.

Recommendation 2: Further guidance to the Annex 1 requirements be developed to aid in its interpretation. The proposed text is presented in Appendix B.

3.2 Recommendations for Methodologies

The group was faced with two problems in confidently evaluating the utility of test methods used in Cycle 1: Only six mills conducted a sensory evaluation, thus, a small database was available. Secondly, although tests employed in Cycle 1 were similar in nature (e.g. triangle tests), it was evident from the review of existing sensory evaluation protocols (NCASI, 1987; ASTM, 1989) that conflicting guidance was offered (e.g. lab exposure regimes, fish acclimation periods), depending upon the protocols followed (sections of the ASTM protocol have been revised since the beginning of Cycle 1). It was also suggested that although some of the methods used in Cycle 1 were useful for some site-specific objectives, they would not necessarily address the overall objectives for EEM (i.e. whether or not the pulp and paper mill effluent is responsible for the observed differences).

Test methods used in Cycle 1 were either inappropriate or inappropriately applied, generated inconclusive results or were overly sensitive. Therefore, the use of objective sensory testing for presence and intensity of taint (as in the difference-from-control test) is recommended for future studies. However, site-specific requirements and objectives may dictate the supplementary use of other sensory evaluation tests, subject to good scientific practice.

Recommendation 3: Further guidance be developed for test methods used, including those for fish exposure and for sensory evaluation. The proposed text is presented in Appendices C and D.

4. Appendix A: Usability of the Resources Expert Working Group

TERMS OF REFERENCE

PURPOSE

The regions, the mills and the consultants have identified difficulties with some of the Environmental Effects Monitoring (EEM) requirements provided under the Cycle 1 "baseline" monitoring program. To address these concerns, the EEM Technical Management Committee is establishing Expert Working Groups to review the results of Cycle 1 evaluation, identify the technical difficulties and provide recommendations on modified or alternative approaches where existing approaches have proven to be unproductive or do not work. These recommendations will provide the basis for the Cycle 2 requirements.

OBJECTIVES

As principal responsibilities the Expert Working Group will ensure that:

- it reviews the concept and purpose of the various requirements under the EEM program,
- it identifies the difficulties encountered in meeting the program requirements,
- it indicates situations where the requirements have been successfully met ,
- it takes into account situations where the national monitoring approach for Cycle 1 has proven to be unsuccessful and provide alternative approaches that will address these site-specific difficulties,
- it provides a report identifying potential alternative approaches and areas requiring further research.

OPERATING PRINCIPLES

The work of the Expert Working Group will follow the following principles:

1. The basis of the recommendations must be driven by Cycle 1 evaluation.
2. That a nationally consistent approach be respected while allowing for flexibility in addressing site-specific issues.
3. The recommendations must follow the EEM program requirement's criteria.
4. The Recommendations must be finalized by April 1, 1997.

PROCESS

Meetings and teleconferences will be scheduled ahead of time but the process should allow flexibility to have meetings when required. As a starting point we suggest an early meeting at the end of June followed by one meeting a month for the following four months. An October meeting at the Aquatic Toxicity Workshop in Calgary should be attempted.

The EWG will be responsible to provide its own secretariat support and will decide on its structure.

PARTICIPANTS

The Expert Working Group (7-8 people) will attend monthly meetings/teleconferences starting at the end of June and extending until the completion of the recommendations at the end of October. Additional individuals may be consulted from time to time on specific issues on an ad hoc basis. In addition to their technical and scientific expertise, participants will be expected, from time to time, to provide the Expert Working Group with secretarial support (eg. taking minutes of meetings, writing sections of the Recommendations Report).

Before committing to these groups, potential participants must ensure that they can attend all of the scheduled meetings and teleconference. A final list of participants will be compiled by the Chair of the working groups.

FINAL REPORT

A report summarizing the Expert Working Group's results and recommendations will be prepared by the Group and distributed to the *National EEM Team*, to the *EEM Technical Management Committee* and to the *EEM Management Steering Group* by April 1, 1997.

TIME PERIOD

October 96 to April 97

Ed Porter
Co-Chair

Valerie Naish
Co-Chair

5. Appendix B: Guidance to the Annex I Trigger Requiring Tainting Evaluation

Because the decision to investigate fish tainting associated with a pulp and paper mill is expected to occur on a mill by mill basis, the local knowledge and expertise of regional authorities is critical for ensuring that there is a sound basis for triggering tainting evaluations. The need for tainting studies should be based on a range of pertinent information including historical information, records of complaints, supporting information associated with a record of complaint, any previous tainting studies that were undertaken, and any subsequent environmental alterations or changes to mill operations that might be relevant.

Thus it is incumbent on the local authorities, taking into account the mill's operating requirements and the interests of all stakeholders, to assess whether a tainting study should be required as part of the current EEM Cycle.

In cases where the responsible parties or the mill ownership do not have immediate knowledge of all prior information from which to assess whether tainting evaluations are required, some effort might be required to substantiate and/or validate existing information. In a similar manner, it might be necessary to confirm that the lack of complaints or documentation pertaining to a tainting problem is not a consequence of the abandonment of previously utilized fisheries resources by local users. A lack of utilization of fisheries resources should be due to tainting and not simply due to the perception that because a mill is in the vicinity it is not worth fishing there. At the same time, a lack of complaints should be due to a lack of tainting and not due to the fact that local users have not utilized the resource for many years because the fish were tainted at some time in the past. In most cases such situations will be readily apparent, but there might be instances where further substantiation or validation is required.

When further substantiation or validation is required, a number of considerations may be necessary. Of primary importance is whether it is possible to assume that fish tainting is attributable to the discharge of mill effluent. Therefore, the siting of the mill discharge, the location of natural and anthropogenic discharges, physical characteristics (e.g. dilution capacity) of the receiving water body, and the location and utilization of fisheries resources near the mill should all be considered. Confirmation of the location and utilization of fisheries resources may require further research. For example, local libraries often have published histories of the region which could assist with understanding the availability and utilization of fisheries resources. Regulatory agency records could also contain relevant information.

In cases where there is a history of complaints, the source and validity of such complaints should be reviewed. Creel records and/or surveys of local fishers can provide some guidance as to the basis of complaints. However, care should be taken to ensure that these records are unbiased and reproducible.

In cases where tainting evaluations have been undertaken in the past, the results and methods employed should be carefully reviewed. Unfortunately, it has been found that fish tainting

evaluation methods have often been incorrectly applied in the past and therefore the results might not be valid. Because of this, it may be necessary to repeat previous evaluations using appropriate methods and panel management procedures. Guidance is provided in this document with respect to the appropriate evaluation procedures. In summary, the decision to initiate a taint evaluation as part of the current EEM Cycle should be based on several considerations. While most situations will be straightforward, there may be situations that require further investigation before a decision can be made. Regional authorities and the local mill's EEM committee are assumed to be best suited to assess whether taint evaluations are required.

6. Appendix C: Guidance for Fish Exposure Conditions and Methods

6.1 Introduction

Review of the Cycle 1 Pulp and Paper EEM data on fish tainting revealed that a wide variety of methods were applied during the collection and/or exposure of fish to effluent. Some of the capture and exposure methods applied were inappropriate for assessing taint in fish tissues using sensory evaluations. This section provides additional technical guidance to that previously provided by Environment Canada (Environment Canada, 1992).

There are three principal methods for obtaining effluent-exposed fish for sensory evaluations. The methods, ranked in order of preference, are as follows:

1. collection of indigenous species;
2. *in-situ* or on-shore exposures; and
3. controlled laboratory exposures.

The most appropriate method to obtain fish for taint assessment will be dictated by site-specific conditions and the species of interest. **Only if the criteria for the preferred method cannot be met should a mill proceed with the next-best method.**

Although all of the first-Cycle EEMs selected at least one of these methods, the lack of standardization in applying the method resulted in data that were inconclusive as to whether or not tainting occurred at the mills that were studied. Failure to follow key procedures for each of these methods will likely result in questionable data and their interpretation.

Particular attention must be made to the following criteria in order to produce scientifically defensible data from sensory evaluations of fish tissue.

6.1.1. Collection of Indigenous Species

This is the preferred method of obtaining fish for sensory evaluations since the fish are likely exposed for extended time periods and the effects that ingestion of tainted prey may have on fish flavour are also taken into account. However, because of critical site-specific requirements it is anticipated that this method will be applied least often. The fish that are used for tainting assessments should either be the species from which the complaints of tainting originated or a species that is commercially or recreationally important.

For this method to be applicable the following criteria must be met:

- Both reference and exposure fish must be collected from the same water body and similar habitat types.
- The same collection methods must be used in both areas. The order of preference would be trap netting, seining, angling, electrofishing, long lining and gill netting. However, only one method should be selected for the capture of all fish.
- Fish in the reference area must be physically isolated (e.g., dam, falls, rapids) from the effluent-exposed population to be assured that there is no mixing of the two groups.
- The mill must be confident that fish collected in the exposure area have been exposed to effluent for a minimum of 48 hours.
- Standard fish measurements, such as weight, length, sex and age must be recorded.
- Six or more fish are **recommended** for pooling per sample to minimize the effects of natural flavour differences that may be attributable to individual; however, the minimum number acceptable for pooling is three fish per sample.

For this method to be applicable the following criteria should be met:

- Fish from reference and exposure areas should be approximately the same size and age and similar sex ratios should be collected from each area.
- Only fish that are still alive at the time of collection should be used for testing.

6.1.2. *In-situ* or On-Shore Exposures

If the above criteria for the use of indigenous species can not be met then the next best option would be to expose fish to effluent with *in-situ* cages or on-shore exposures. If this exposure method is selected the species from which the complaint of tainting originated should be used in the exposures. However, the appropriateness of this method and the species that is ultimately selected for exposure will be determined by site-specific conditions and the ability to meet the following criteria.

For this method to be applicable the following criteria must be met:

- Fish for the exposures must be collected preferably from a reference area within the mill's receiving environment. Alternatively, fish can be collected from another water body in the area, provided that all fish used for the study are captured in the same water body.
- The same collection methods must be used in both areas. The order of preference would be trap netting, seining, angling, electrofishing, long lining and gill netting. However, only one method should be selected for the capture of all fish.
- Fish must be exposed to effluent for a minimum of 48 hours.

- Six or more fish are **recommended** for pooling per sample to minimize the effects of natural flavour differences that may be attributable to individual; however, the minimum number acceptable for pooling is three fish per sample.
- Standard fish measurements, such as weight, length, sex and age must be recorded

For this method to be applicable the following criteria should be met:

- All fish should be similar in size and age and the sex ratio should be the same at upstream and downstream locations.
- *In-situ* cages or on-shore exposure tanks should be placed in areas where indigenous fish are suspected of being tainted.
- Exposures should be conducted at the time of year when complaints of fish tainting are most common.
- Fish can be acclimated in dilution water for up to ten days, if practical, given the site-specific conditions. However, fish exposures can be undertaken with fish that have not been acclimated provided reference and exposure fish receive the same handling and are obtained from the same water body.
- If the species of interest is unavailable or will not survive in captivity, surrogate species such as rainbow trout may be substituted.

6.1.3. Controlled Laboratory Exposures

The third fish exposure option would be exposure in the laboratory. This option should be selected when site specific conditions would prohibit the use of indigenous species or *in situ*/on-shore exposures.

Rainbow trout are recommended as the surrogate species to be used in laboratory exposures. Trout have been demonstrated to rapidly accumulate off-flavours which can be readily distinguished by sensory panels because of the mild inherent flavour characteristic of the fish flesh. These fish are available from piscicultures across Canada and therefore could be used as a standard species for laboratory tainting evaluations.

Test solution replacement in the laboratory assay can be either continuous flow (with sufficient flow through to maintain 60% saturation of oxygen in the test solution) or static renewal (with solution replacement every 24 hours).

Twenty-four hour composite samples of the effluent to be tested are recommended although grab samples would be acceptable for site specific situations. Fresh samples should be collected each day and transported to the laboratory in clean non-toxic containers (e.g. polyethylene or polypropylene) that are filled to the top so there is minimum headspace. Transportation and storage conditions should be the same as for effluent samples collected for routine acute toxicity tests.

The dilution water for the laboratory exposures (both effluent and control exposures) should be the receiving water from the reference site for the mill, where it is practical. Otherwise the laboratory culture water should be used.

For this method to be applicable the following criteria must be met

- Fish must be exposed to effluent for a minimum of 48 hours;
- The number of fish per exposure must conform with the sample size (# of grams) required for the sensory evaluation. Minimum number of fish test per solution would be 3.

For this method to be applicable the following criteria should be met

- All fish should be within the same size range.
- Maximum loading density of fish in each test concentration should be no more than 10g/L/d.
- Fish used in tainting evaluations should have undergone a period of at least 10 days acclimation prior to the test.
- Fish should not be fed for 48 hours prior to the test in order to minimize off-flavour from the commercial food.
- Lighting should be maintained under the standard conditions recommended for fish culturing.
- There should not be any pH adjustment of effluent or dilution (receiving) water.
- Upon preparation, pre-aerate each test solution at ≤ 7.5 ml/min for a maximum of 90 minutes or achieving 70% saturation in the highest concentration. The test solutions should be aerated at this rate throughout the static renewal test.
- Test solutions should be maintained within $\pm 2^\circ\text{C}$ of the acclimation temperature.

6.2 Fish Capture, Preparation and Preservation in the Field

The goal of these methods is to stabilize harvested fish immediately to prevent any changes due to quality deterioration which could interfere with the evaluation of flavours from exposure to effluent. The important factors are cleaning, protecting from exposure to air, and chilling (first by holding on ice, then by freezing as soon as possible).

Under no circumstances should the viscera come into contact with the flesh to be used for sensory evaluation. Ideally, fish should be cleaned by eviscerating and removing kidney and gills and washing in potable water (such as distilled water taken to the harvest site for this purpose) immediately after harvesting. Following that, fish should be:

- Placed in tub or plastic wrap on flake ice, belly cavity down to allow drainage. Cover with plastic wrap and ice and transport to processing lab as soon as possible. Note: if fish are to be transported on ice to a processing facility before cleaning, the round fish

should be placed belly up to reduce the pressure on the contents of the belly cavity and prevent internal rupture.

- Wrapped in foil or plastic wrap to shield from air. Place in single layer on trays and freeze solidly as soon as possible.
- When frozen, vacuum seal in plastic bags, then in master container (cardboard or plastic) and return to frozen storage. Samples should be held at least at -18°C but preferably lower. The lower the temperature of frozen storage, the more stable the samples will be. For long term storage of samples, the use of jacketed cold rooms with no automatic defrost or holding at extremely low temperatures, e.g. -60°C is highly recommended. During shipping, samples should be maintained in the frozen state to prevent any exposure of fish to air, etc.

Note: Culling fish during field sampling may be necessary under certain conditions, but should be limited to specific conditions and should be documented. Fish which have died before harvesting and those showing obvious signs of disease should not be used. Culling must be documented. Also, during sampling, records should be made, as supplementary information, of fork length, weight, sex for all samples taken, and efforts should be made to maintain similar sex ratios at all sampling site. Balance male and female fish from each sampling site.

6.3 Fish Preparation in the Laboratory

The goal of these methods is to prepare fish for serving to sensory panel without causing quality changes during the handling of raw fish, flavour changes due to cooking and changes due to loss of volatiles by subsampling cooked fish. The important points here are:

- Thawing fish is done under refrigeration only until thawed enough to handle. As above, if fish have been frozen round (without cleaning), they should be thawed only enough for the belly cavity flesh to be cut and the still-frozen viscera removed intact. Kidney should be washed away immediately.
- When filleting and skinning, care should be taken to keep the flesh away from any slime, scales, etc. from the skin and to keep the fillets chilled during handling.
- Blend fillets to create homogeneous samples for each treatment/replication. Composite samples are necessary to reduce the effect of individual variability in uptake of effluent into the fish, as well as fish to fish variability. The number of fish that should be used will depend on:
 - the size/weight of the individual fish and the total amount necessary for each replication of the sensory test - different lots of fish will be blended for each replication
 - a minimum of 6 fish should be used.²

² In work at the Department of Fisheries and Oceans on muddy-earthly off-flavours and using rainbow trout of approximately 200g dressed weight, 6 fish were used per sample source per replication. In work on channel catfish, "fillets from all fish of an experimental sample" were used to prepare the blended sample. (York, R. personal communication and Johnssen, P. & Kelly, C.A. 1990.)

- Condiments such as salt, etc. are not added to the fish as they may mask flavours present in the fish.
- Individual samples are wrapped in heavy aluminum foil and coded with unique (for each treatment/replication) three-digit codes. This preparation format allows the samples to be completely sealed for cooking and does not require subsampling after cooking. This means that the assessor is able to evaluate all the volatiles present in the fish sample. (ASTM E-1810-96)
- The individual samples contain from 7 to 15 g of fish, depending on available fish and number of samples required.
- Samples are cooked in a steamer for seven minutes if samples are thawed or 12 to 15 minutes if samples are frozen. Separate steamers must be used for control and experimental fish. The packets are not unwrapped during or after this preparation step. Steaming prevents the addition of flavours from the cooking process, such as those due to browning.

Note: Culling fish during laboratory preparation should be limited to removing fish which show signs of quality deterioration e.g. freezer burn. Culling must be documented. Also, during sampling, records should be made, as supplementary information, of fork length, weight, sex for all samples taken, and efforts should be made to maintain similar sex ratios at all sampling site. Balance male and female fish from each sampling site.

7. Appendix D Guidance for Sensory Evaluation Panels and Methods

7.1 Introduction

Sensory analysis is used to measure the presence and intensity of taint. The definition of tainted fish used here is based on the ISO 5492:1992 Sensory Analysis - Vocabulary and is "fish that contains an abnormal odour or flavour". The context of the definition is that the odour or flavour is introduced into the fish from outside sources and does not include any natural by-product of the deterioration in quality due to aging of fish in storage, decomposition of protein, fat, or other components, or of microbial contamination normally found in fish.

The purpose of this section is to describe sensory test procedures to measure the presence and intensity of taint in fish exposed to pulp mill effluent in either field or laboratory conditions. The following information describes standard methodologies for the selection, training and use of sensory panels for these objective assessments.

It is important to select appropriate test methods to measure the success, or lack thereof, of treatment changes on the resulting effluent and the impact on fish populations. The following is a brief review of the test methods used previously in Cycle 1 and the difficulties which arose from their use, and a review of the recommended test method for Cycle 2.

7.1.1. Triangle Test (Forced Choice Test)

Some key points regarding triangle tests are given in the ASTM Draft Standard - Standard Test Method, Sensory Analysis - Triangle Test, December 1996. These are particularly important to the assessment of taint:

- the procedure is used to determine whether any perceptible sensory difference exists between samples of two products and any difference, not only the presence of taint will be used by the assessor in examining the samples.
- The difference may be in a single sensory attribute or in several.
- The test is applicable when the nature of the difference is unknown, but it does not determine the size or direction of difference and without training it does not identify the attribute responsible for the difference
- It is applicable only if the products are homogeneous, i.e. treatment and control samples must be taken from the same water source, and be exposed to exactly the same condition with the one exception being the presence of a taint source.
- As a relatively large number of samples will be required, it is applicable when the products do not cause excessive sensory fatigue, carryover, or adaptation. As in other sensory tests, experience and familiarity with the product may increase the sensitivity of the assessor and increase the likelihood of finding a significant difference, whether or not it is due to the presence of a taint.

- As in other sensory tests, assessors are chosen in accordance with the test objectives - to increase protection of product quality, assessors with demonstrated acuity should be selected. This has not been the case in the tests reported in Cycle 1.

7.1.2. Preference/Acceptance Testing

Preference and acceptance testing are subjective tests used for determining consumer preferences for and attitudes towards different products. They are designed to be used with untrained assessors and measure opinions and biases rather than product characteristics. In this type of testing, there is no right or wrong answer, as any like/dislike response to the sample is valid. This creates a problem in the interpretation of data regarding the quality of the samples, i.e. the presence or absence of taint. A prime example of the fallacies of using this type of testing for measurement of quality in fish is given by Kosmark (1987).

Another consideration in Preference/Acceptance testing is the sample size required for data collection. Panel sizes range from 50 to several hundred assessors.

7.1.3. Difference-From-Control Test

The difference-from-control test is recommended for Cycle 2 of the EEM program for the evaluation of taint in fish flesh. This test method is often used in quality control situations for the measurement of a difference and the estimation of the size of any differences found. The advantage of this method over tests such as the triangle test for this situation is that the nature of the difference required is easier to define, i.e. taint, and the assessor is less influenced by other factors in the sample. The data gathered can be analyzed through standard statistical tests to give measurements of intensity of taint relative to the control samples. The tests are completely described in Meilgaard et al (1991) and Munoz et al. (1992) and are given in section 5.1.3.

7.1.4. Recent changes to ASTM D3696-95

A new ASTM standard E1810-96 *Standard Practices for Evaluating Effects of Contaminants on Odour and Taste of Exposed Fish* has been published by Committee E-18 on Sensory Evaluation of Materials and Products. This standard supersedes the sensory evaluation procedures given in D3696-95 *Standard Practice for Evaluating an Effluent for Flavour Impairment of Fish Flesh*. (Standardization News, January, 1997, p. 4)

7.2 Recommendations for Sensory Evaluation of Taint

7.2.1. Personnel Requirements

7.2.1.1. Personnel Conducting Sensory Evaluations

All of these sensory evaluation methods have test protocol requirements which make it essential that they be conducted by or supervised by a person with specific training in sensory analysis, especially in panel selection and training and in test design and analysis. This qualification should be demonstrated by university level training at the graduate level or by undergraduate training with employment experience. In both cases, evidence of professional standing is also appropriate.

7.2.1.2. Participating Assessors

The selection of assessors who have the basic sensory acuity to perform the tests needed is essential to the collection of reliable data. **Note: Pregnancy is one factor which will automatically eliminate a candidate, as may other health factors (e.g. allergies, sinus problems, some medications) that could interfere with sensory acuity.** When working with samples which may have carry-over effects, it is essential to select assessors who are able to use the techniques available to reduce these effects in the testing sessions. A recommended method for assessors selection is given in Section 7.2.4.1.

The personnel selected for participation in sensory evaluation panels should be trained and validated in the collection of objective sensory data for the presence and intensity of taint in fish using the methods given in Section 7.2.4.1. This includes training in the use of the test methods which will be used for data collection, and instructions on the use of the ballots, sample handling during assessments, rinsing procedures, etc. They are also given experience with known samples of tainted and control fish, and their ability to participate in the test is further assessed through these samples.

7.2.2. Facility Requirements

All sensory tests should be conducted in an appropriate sensory testing facility which includes:

- adequate and appropriate lighting for the task
- proper ventilation which ensures removal of odours from the testing area
- control of noise and distractions during testing.

It is recommended that sensory tests be conducted in a test facility which conforms to the requirements of ASTM STP 913 *Physical Requirement Guidelines for Sensory Evaluation Laboratories* (1986).

7.2.3. General Requirements for Sensory Evaluation Panels

7.2.3.1. Panel Management (QA/QC)

- Quality control of the sensory panel includes the tracking of individual panel results to assess any changes in sensory acuity. If this occurs, retraining is necessary. This can be assessed through data analysis such as the Analysis of Variance, where the main effects become: Samples, Panelists, Replications. The inclusion of panelists in this allows the determination of the consistency with which the individuals rate the samples as well as their consistency across replications.
- Part of the test design for assessment of pulp mill effluents includes the addition of known control samples or standards to the test samples. Known control samples also allow the monitoring of the panel to be measured during test sessions.
- It may be necessary to replace panel members, if any withdraw from the panel. In this case, it will be necessary to repeat the selection procedures and the initial training panels for the new member(s).

7.2.3.2. General Procedures for Sensory Panel Sessions

The following points are common to all of the sensory test methods described in this document.

- Prior to entering the panel room, the panelists should wash their hands with odourless soap and rinse to remove any trace of residue. During panel sessions, panelists should clean hands of any fluid residue from samples using a paper napkin dipped in odour-free water - to prevent cross-contamination of samples and physical carryover of any stimuli.
- Rinsing between samples using neutral materials is standard practice during sensory evaluation sessions. The purpose of rinsing is to prevent the carry-over of stimuli from one sample to the next. Odour-free water (e.g. distilled) is recommended along with unsalted soda crackers. When crackers are used, they must be followed by a water rinse to remove any residue from the mouth. It has been found that using odour-free water heated to 50°C is useful in removing flavours between samples. In ASTM D3696-95, the recommendation is still given to use a dilute lemon juice rinse during panel sessions. **DO NOT USE LEMON JUICE RINSE** as it can interfere with low levels of sourness in the samples.
- Time between samples is required to avoid carry-over of odour/flavours from the previous sample. A minimum of one minute should be taken between samples, however, a longer time may be needed in specific testing conditions. Panelist fatigue is minimized by rinsing and also by limiting the number of samples which are analyzed at each session. The number which can be tested will be established during training. If there is a strong carry-over effect, 1-3 treatments with appropriate rinsing may be the limit for number of samples per session. If carry-over effects are less, then a larger number of samples can be evaluated, with appropriate rinsing, usually not exceeding 8-10 per session.
- The panelists' instructions for handling samples during evaluation sessions include:
 - open sample packets by tearing off one folded end and down one side of the foil.

- evaluate the odour of the samples first then close the packet temporarily.
- to taste, divide the sample in half and do the assessment. Use second half as needed for re-tasting.
- rinse after each sample set.
- Panel methodology for the analysis of taint includes the use of "warm-up" samples at the beginning of each test session. This allows the sensory systems to "re-experience" the sensory attributes which will be evaluated before the actual test samples are begun. Data may be collected on these samples, however it is not used in the final analysis of the study.

7.2.4. Recommended Sensory Evaluation Methods for Cycle 2

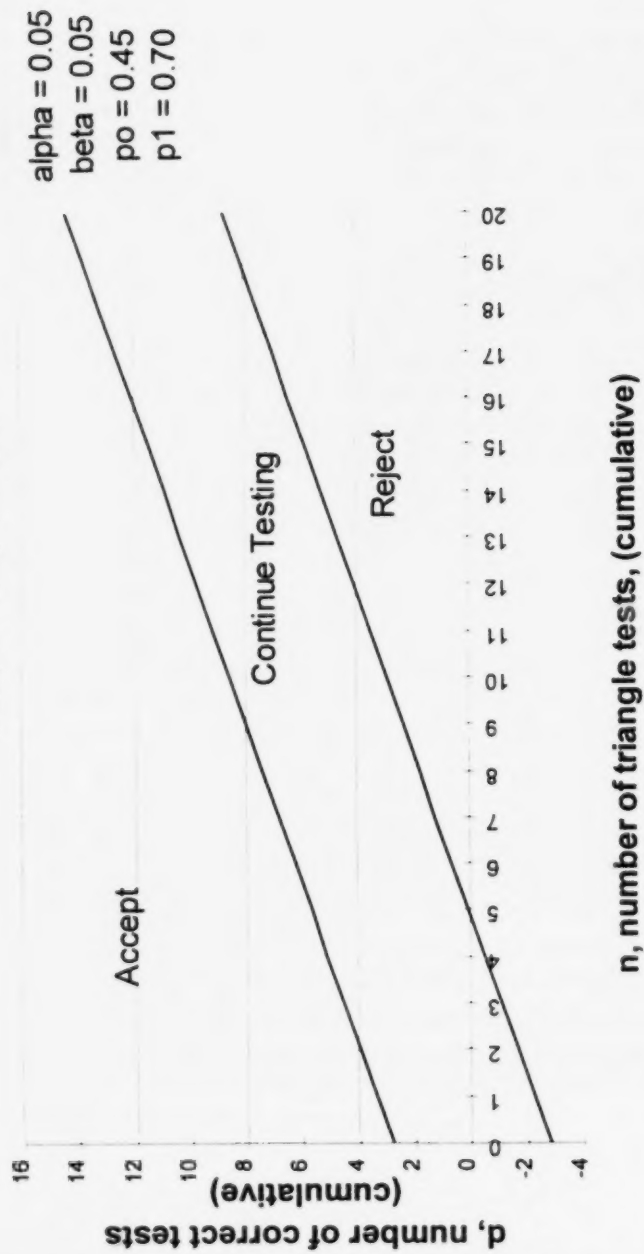
The following sections provide information on the sequence of appropriate sensory methods for objective sensory evaluation of fish taint from pulp and paper effluent.

7.2.4.1. Assessor Selection by Testing for Sensory Capabilities using "Sequential Testing"

The purpose of these procedures is to select panelists with the sensory abilities to perceive the sensory attributes for which the samples are being examined and the personal qualities to participate in the tests in a consistent and professional manner.

The group of tests which fall under the heading of "sequential testing" are actually difference tests which are used with preset statistical criteria. They allow the decision of "accept for panel", "reject for panel" or "continue testing" to be made through the use of a pre-set graphical representation of the statistical criteria (Fig.1). These tests have been described in the sensory literature (Amerine et. al. (1965), Meilgaard et al. (1991) and Munoz et al. (1992)) as being meant to "economize" on the number of tests which must be conducted in order to accept or reject a panel trainee while providing a statistical basis on which to base this decision. This method is based on the *a priori* selection of the statistical levels of α & β probabilities for the level of performance of the assessors and the use of difference tests such as triangle, duo-trio, and paired comparison-difference.

**Figure 1: Graph for Sequential Analysis for Selection Panel Trainees
Using Triangle Tests**



7.2.4.1.1 Samples for Selection of Assessors

Samples which can be used for assessor selection include:

- tainted fish from field or laboratory sampling.
- an appropriate model system using either fish flesh or water as the carrier and appropriate dilutions of critical compounds identified in the pulp and paper mill effluent or appropriate dilutions of the effluent itself.
- the concentrations chosen should represent a level of difficulty appropriate for the effluent tested. While these concentrations will be set on a mill by mill basis, they should represent relatively small differences so that the selected panel will demonstrate the ability needed to collect useful data.

7.2.4.1.2 Test Design

The test methods used for selection of assessors to ensure that the panel will consist of individuals who are able to perceive the odours/flavours of effluent taint are difference tests such as triangle, duo-trio, and paired comparison-difference. This is a situation in which the triangle test can be used effectively, as there is adequate control over the nature of the treated and the control samples. The recommended procedure included is the triangle test used in the format of the 3-alternative forced choice method. In this form, the three samples of the test always include two control and one treated sample. This is done to decrease carry-over effects and to increase the chance of being able to detect the different sample.

Sequential testing requires the calculation of control graphs to evaluate the performance of each of the assessors. The criteria used in calculating the control graphs (Fig. 1) are based on the type of test used and the levels of statistical significance chosen. The recommended levels of statistical significance are $\alpha=0.05$ & $\beta=0.05$.

7.2.4.1.3 Test Method

The test method consists of the presentation of appropriate sample sets under appropriate testing conditions as described in Sections 7.2.2, 7.2.3.1 and 7.2.3.2.

The sample sets are presented in the format of the triangle test and the resulting correct/incorrect responses plotted on Figure 1. The samples to be used must be established for the concentration(s) of stimulus which will be used. As a triangle test is being used, the samples used in selection must be homogeneous in nature and be demonstrated to contain no other differences except for the tainting mixture.

If other tests are used, such as the Duo-Trio test or the Paired Comparison Test, control graphs must be calculated for these tests using the methods described by Amerine et. al. (1965), Meilgaard et al. (1991) and Munoz et al. (1992).

7.2.4.1.4 Personal Suitability Interview

This is conducted to assess the personal qualities of the candidates who have been selected to participate in the panel to ensure that they will be able to conduct sensory assessments in a consistent and professional manner. As stated previously, pregnancy is one factor which will automatically eliminate a candidate, as may other health factors (e.g. allergies, sinus problems, some medications) that could interfere with sensory acuity.

During the personal suitability interview, the candidate is assessed for such characteristics as:

- interest in panel work and in the particular study.
- availability.
- cooperativeness (e.g. willingness to refrain from using any perfumes or other scented lotions) as these may interfere with the individual's performance and will be a distracting odour to other members of the sensory panel, for the duration of the sensory tests.
- general health (chronic health problems which could interfere with sensory panel performance: allergies, chronic nasal problems, medications, etc).
- There is no limitation on age of panelists, as the ability to perform the test is assessed during the first stage and the ability to perceive odour is more dependent on individual ability than on general age.

7.2.4.2. Training of Assessors

This step is essential to establish a calibrated panel and should be expected to take between one to three weeks of panel sessions.

The training program includes several sessions during which the assessors become trained to:

- use the correct procedures from the required test method:
 - sample handling.
 - evaluation techniques to reduce fatigue - rinsing, time, etc.
 - analytical approach to testing and disregarding personal preferences.
 - correct usage of the ballot for recording data.
- accurately evaluate the actual samples through the presentation of known, prepared samples which are representative of the range of concentrations which will occur in the final test:
 - begin with large, easily perceived differences.
 - progress to smaller but easily perceived differences.
 - continue until assessors can demonstrate successful "calibration" (data analysis here can be ANOVA comparing treatments and panelists as main

effects and use the treatment x panelist interaction to measure the overall performance of the panel).

- in studies which use fish tainted in the laboratory, samples of fish which have not been cleared of feed flavour can be presented to the assessors to demonstrate the difference between any potential residual feed flavours and actual taint flavours.

Training calibrates the panel and provides the individual assessors with confidence in their sensory abilities required to perform the test.

Subsequent taint studies which use the same panelists do not generally require the same amount of time for training as does the initial panel. In this case, the training acts as a "refresher" and re-calibration for the next data collection panels.

7.2.4.3. Difference-From-Control Test

In the Difference-from-Control Test, samples of treated fish which have been exposed to pulp and paper mill effluent either under field or laboratory conditions and known control samples (standards) are compared with an identified control sample. The assessment of the presence of a difference based on a specified characteristic, i.e. taint, and the degree of that difference are made using one of the ballots shown in Figures 2 and 3 (either the unstructured line scale or the category scale will be used consistently within each study). Details of this test method are given in Meilgaard et al. (1991) and Munoz et al. (1992).

Number of measurements required: using a panel of 10 to 12 selected, trained individuals, one measurement per sample per judge per replication is taken. A minimum of three replications should be conducted per study. The blind control samples are inserted into the test design to measure the "background noise" or placebo effect.

The responses from the unstructured line scale can be decoded into numerical data using either a six-inch grid with 10-to-the-inch divisions (with readings taken as x.x) or a ruler with suitable divisions (15 cm with readings taken as x.x). Responses for the category scale are recorded as values from (0 to 9). Data collection for each replication includes the recording of the values for each of the sample treatments given by each of the panelists so that individual assessor's data can be tracked and the panel performance and consistency assessed across replications and treatments.

Data analysis include the conducting of appropriate statistical analyses, such as analysis of variance (and multiple comparison difference tests where significant differences are found) for the evaluation of the treated samples relative to the control samples across replications and assessors.

Note: Using trained panels and proper rinsing procedures, it is possible to present multiple coded samples with the one reference sample.

Figure 2. FORMAT OF BALLOT FOR DIFFERENCE FROM CONTROL - using the unstructured line scale.
(Meilgaard et al.(1991):

DIFFERENCE FROM CONTROL TEST

Name: _____

Date: _____

Type of Sample: _____

Test # _____

Attribute assessed: Presence of TAINT or OFF-ODOUR OR OFF-FLAVOUR

Instructions:

1. Taste the sample marked "Control" first.
2. Smell/taste the sample marked with the three digit code. Do not swallow the sample, expectorate any sample which is tasted.
3. Assess the overall sensory difference between the two samples using the scale below.
4. Mark the scale to indicate the size of the overall difference by placing a vertical mark across the horizontal line at the point that best describes the degree to which the sample deviates from the reference.

Code No. _____

_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R
_____	same as R	_____	extremely different from R

(Remember that a duplicate control is the sample some of the time.)

COMMENTS:

Figure 3. FORMAT OF BALLOT FOR DIFFERENCE FROM CONTROL - using the category scale. (Meilgaard et al.(1991):

DIFFERENCE FROM CONTROL TEST

Name: _____

Date: _____

Type of Sample: _____

Test # _____

Attribute assessed: Presence of TAINT or OFF-ODOUR OR OFF-FLAVOUR

Instructions:

1. Taste the sample marked "Control" first.
2. Smell/taste the sample marked with the three digit code. Do not swallow the sample, expectorate any sample which is tasted.
3. Assess the overall sensory difference between the two samples using the scale below.
4. Mark the scale to indicate the size of the overall difference by placing a vertical mark across the horizontal line at the point that best describes the degree to which the sample deviates from the reference.

Code Number	_____	_____	_____	_____
No difference 0	_____	_____	_____	_____
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____
5	_____	_____	_____	_____
6	_____	_____	_____	_____
7	_____	_____	_____	_____
8	_____	_____	_____	_____
Extremely different 9	_____	_____	_____	_____

Code Number	_____	_____	_____	_____
No difference 0	_____	_____	_____	_____
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____
5	_____	_____	_____	_____
6	_____	_____	_____	_____
7	_____	_____	_____	_____
8	_____	_____	_____	_____
Extremely different 9	_____	_____	_____	_____

(Remember that a duplicate control is the sample some of the time.)

COMMENTS:

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